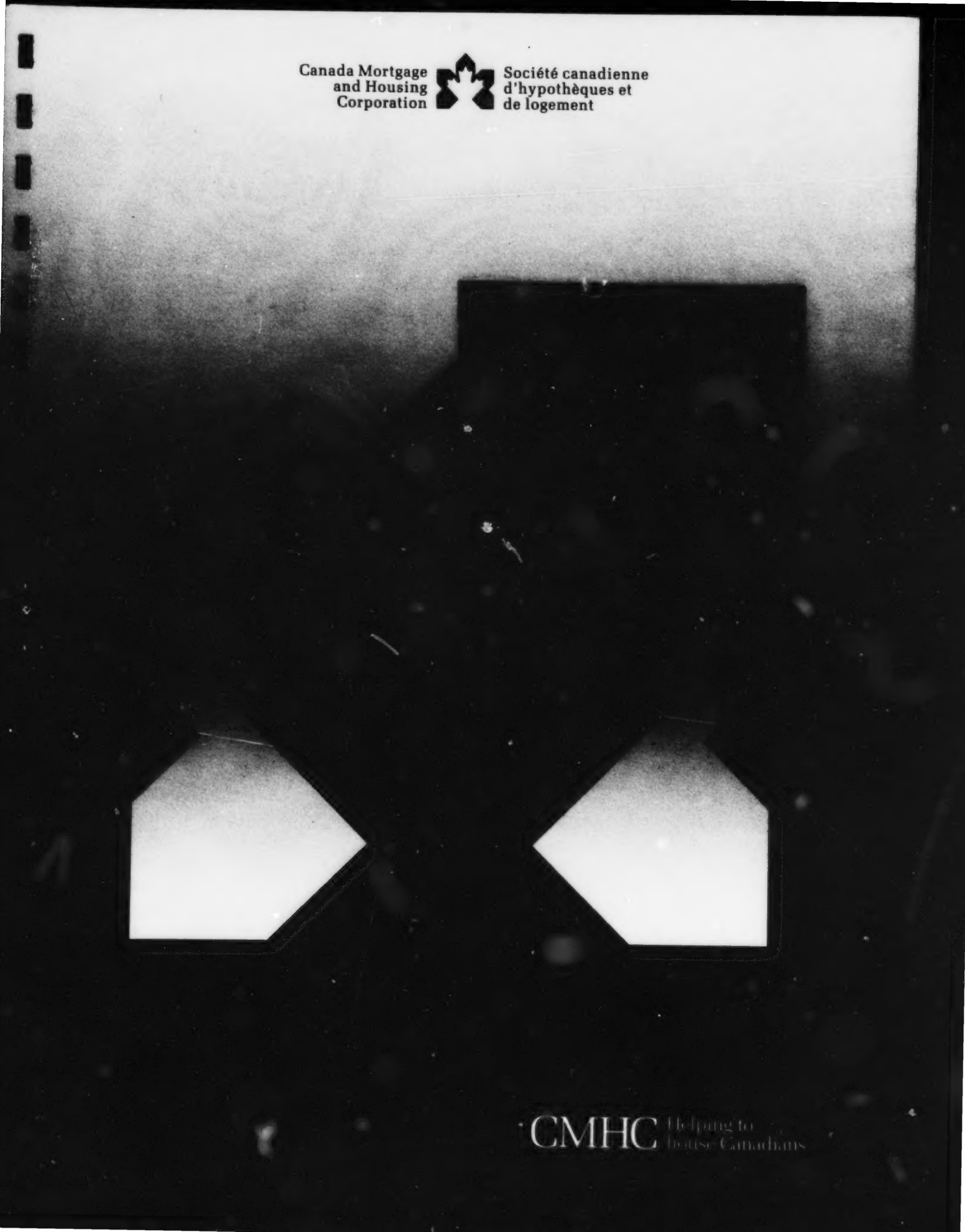
Canada Mortgage and Housing Corporation
Société canadienne d'hypothèques et de logement



CMHC Helping to house Canadians

CALIBRATION OF THE RCS SAMPLER

PROJECT REPORT

Prepared for:

Mr. Jacques Rousseau
Project Manager
Project Implementation Division
Canada Mortgage and Housing Corporation
682 Montreal Road
Ottawa, Ontario
K1A 0P7

Submitted by:

Paracel Laboratories Ltd
8-17 Grenfell Crescent
Nepean, Ontario
K2G 0G3

(613) 225-2447

August 5, 1988

Canada Mortgage and Housing Corporation, the Federal Goverment's housing agency, is responsible for administering the National Housing Act.

This legislation is designed to aid in the improvement of housing and living in Canada. As a result, the Corporation has interests in all aspects of housing and urban growth and development.

Under Part V of this Act, the Government of Canada provides funds to CMHC to conduct research into the social, economic and technical aspects of housing and related fields, and to undertake the publishing and distribution of the results of this research. CMHC therefore has a statutory responsability to make widely available, information which may be useful in the improvement of housing and living conditions.

This publication is one of the many items of information published by CMHC with the assistance of federal funds.

Collection Efficiency of the RCS Biotest Sampler

The objective of this research was to establish whether the RCS sampler collected spores of different fungi with equal efficiency and estimate whether the collection process was correct in absolute terms. Fungal spores in indoor air pose different health hazards, and spores are greatly variable with respect to size, density, and adhesivity. There will be intrinsic and extrinsic flaws in all spore collection devices (1), but it is neccesary to know if, for example, a spore collection device significantly "under collects" species with small spores (3-4 microns) versus those with larger spores (e.g., 50-100 microns) or vice versa. A number of studies have been done wherein different spore collection devices (e.g., Reuter Centrifugal Sampler (RCS), slit-to-agar, Anderson) have been compared side by side (2,3,4,5,6). Unfortunately, the variablity of spores in indoor air is extreme because spores are often introduced as pulses from the source ("amplifier"). Thus, the results of such studies have been contradictory and hence, inconclusive. For example, Placencia et al. (5) concluded that the RCS sampler collected bacteria more efficiently than a slit-to-agar sampler. Clark et al. (6) concluded that RCS samplers were not the samplers of choice in hospital settings. Macher and First (7) modified a Reuter centrifugal sampler made by Mattson-Garvin (model 200) in an attempt to measure the collection using aerosols in water of polystyrene latex spheres. These authors claimed that particles with a diameter of less than 6 microns were not collected efficiently. These experiments only added to the contradictions on this matter because the results contradict more than half of the studies (including the present study) involving viable particles. The extensive modification of the air inlet geometry could have produced artifacts and adequate control experiments were not reported to judge this.

The assessment of particles in air is a well established science. NIOSH has evolved a number of standard protocols for this and for the testing of particle counting protocols, a

particle generating chamber has been described which is available commercially (8). A modification of this device has been reported in studies of aerosolized fungal spores (9). This study reports the further use of a fungal spore generator/chamber suitable for the insertion of an RCS sampler or particle counter probe for the purpose of evaluating the collection efficiency of the RCS sampler manufactured by Biotest Diagnostics (Biotest-Serum-Institut Gmb, Frankfurt, FRG) of spores from 4 to 60 microns in size together with an estimate of the absolute efficiency of the device. The common phylloplane fungi Cladosporium cladosporioides (spores (3-7)x(2-4) microns), Alternaria alternata ((20-63)x(9-18) microns), and the toxigenic fungi Paecilomyces varioti ((2.5-3)x(5-7) microns) and Penicillium viridicatum (3-4 microns) were used.

Materials and Methods

The spore generator/chamber was constructed after Sorrensen et al., (9; Fig. 1). The entire apparatus was maintained in a controlled environment chamber at 25^{o}C, and the temperature and humidity were monitored. These experiments required considerable trial and error to determine the optimum voltage, frequencies, air flows, spore concentrations, etc., and these experiments are not described. Spores were collected from four species of fungi using two methods. In the case of Cladosporium cladosoporiodes, Paecilomyies varioti and Penicillium viridiratum, the procedure was as follows. Each strain was innoculated on sterile rice (5g) to which 1 mL of water had been added. These were incubated for 7-10 days at 25^{0}C. After incubation, the rice cultures were shaken with sterile water containing Tween 20 (0.01%) for 30 minutes, filtered through Whatman # 2 filter paper, and dried under vacuum for 48 hr at 40^{0}C. In the case of Alternaria alternata, the strain used was grown on moistened filter paper for up to three weeks. The filter paper was shaken in sterile water/Tween 20 solution as above and filtered through cheese cloth. This was filtered a second time with Whatman # 2 filter paper and the spores dried as above.

Small spore masses were used for these experiments, keeping within the useful range of the RCS sampler (i.e., 0.05 to 50 ug). Silica gel (0.063-0.2 mm) served as the dilution agent in experiments with the RCS sampler. A given mass of pure fungal spores was diluted by weight, yielding the spore masses indicated in the figures. To ensure this was done properly, each diluted sample within a series for a given strain was plated by dilution plating on 2% malt extract agar, in triplicate, over several dilutions (i.e., 10^{-4}, 10^{-5}, 10^{-6}, etc.,) as appropriate and CFU g^{-1} silica mixture determined. In most cases, the dilution was satisfactory, and there was little or no difference between the expected and actual results (see results).

In the case of the chamber verification studies, spores of C. cladosporioides only were used. A Royco particle counter (model 218) was set to record all particles greater than 2 um, so a larger particle size diluting agent (small glass beads) was utilized so as not to excessively interfere with the spore counts.

The tests were carried out under the following conditions. The apparatus was set up as shown (Fig. 1). The spore mixture to be tested was placed at the bottom of the surface sterilized chamber and the sampler placed through the rubber seal and fixed to the stand. The voltage and frequency were set and measured by the oscilloscope to be 2 V at 132 Hz. An air flow of 8 L min^{-1} was passed through the tube during the sampling time of the RCS (30 s). The Biotest RCS was operated according to manufacturer's instructions. Each measurement was taken several times. The RCS strips were incubated at 25°C for 3 to 5 days and CFU.

In the case of the Royco counter, a series of blank glass bead measurements were done before the spore/glass bead mixture was tested. These were then subtracted to get a count reflective of the spores only. The experiments with the Royco particle counter had to be done with spore concentrations exactly 100 times that used for the RCS sampler. The air intake of the Royco instrument was much lower than that of the RCS (283 mL min^{-1} versus 40 L min^{-1}).

Results

The data are plotted on Figures 2, 3, 4, and 5. The data (1) indicate that, within the useful range of the RCS sampler, the collection efficiency is reasonably linear and (2) on the basis that the slopes of the lines are similar with an average percentage standard error of ea. 10%, disregarding C. cladosporioides, that the large spores of A. alternaria are collected with approximately the same efficiency as those of Penicillium viridiratium. Some of the apparent deviation, particularly in the case of C. cladosporioides, is probably due to loss of viability of the spores between the time the RCS measurements were taken and when the dilution platings were done. Unfortunately, this was not anticipated.

The data generated with the Royco particle counter with spores of C. cladosporioides (Fig. 6) also demonstrated that the generation of a spore suspension within the chamber was reasonably linear. Additionally, and taking into account the different absolute amounts of spores used compared to the RCS measurements of C. cladosporioides (Fig. 5), the RCS CFU m^{-3} values are very similar. This indicates that the value obtained by the RCS is comparable to the absolute value over the useful range of the device. Again, this is sustained by the result for Penicillium viridicatum wherein the slope is nearly 1:1.

Conclusions

These experiments have demonstrated that the RCS sampler collects spores of greatly different sizes and shapes with approximetely equal efficiency and that the CFU m^{-3} value obtained can be predicted to be similar to the absolute value over the useful range of the sampler (0-2000 CFU m^{-3}).

Figure 1 (opposite page)

1. Sine-wave generator, Esterline Angus, Model F-1000
2. Amplifier, Lifco
3. Loud speaker, 20.5 cm diameter
4. Oscilliscope, Tektronix 2465, 300 MHz
5. Plexiglass tube, height: 37 cm, diameter: 25 cm
6. Dental rubber sheetings
7. Flow meter, Gilmont
8. Air purifier, Koby
9. Hygrothermograph, Cole Parmer
10. RCS Biotest Sampler.

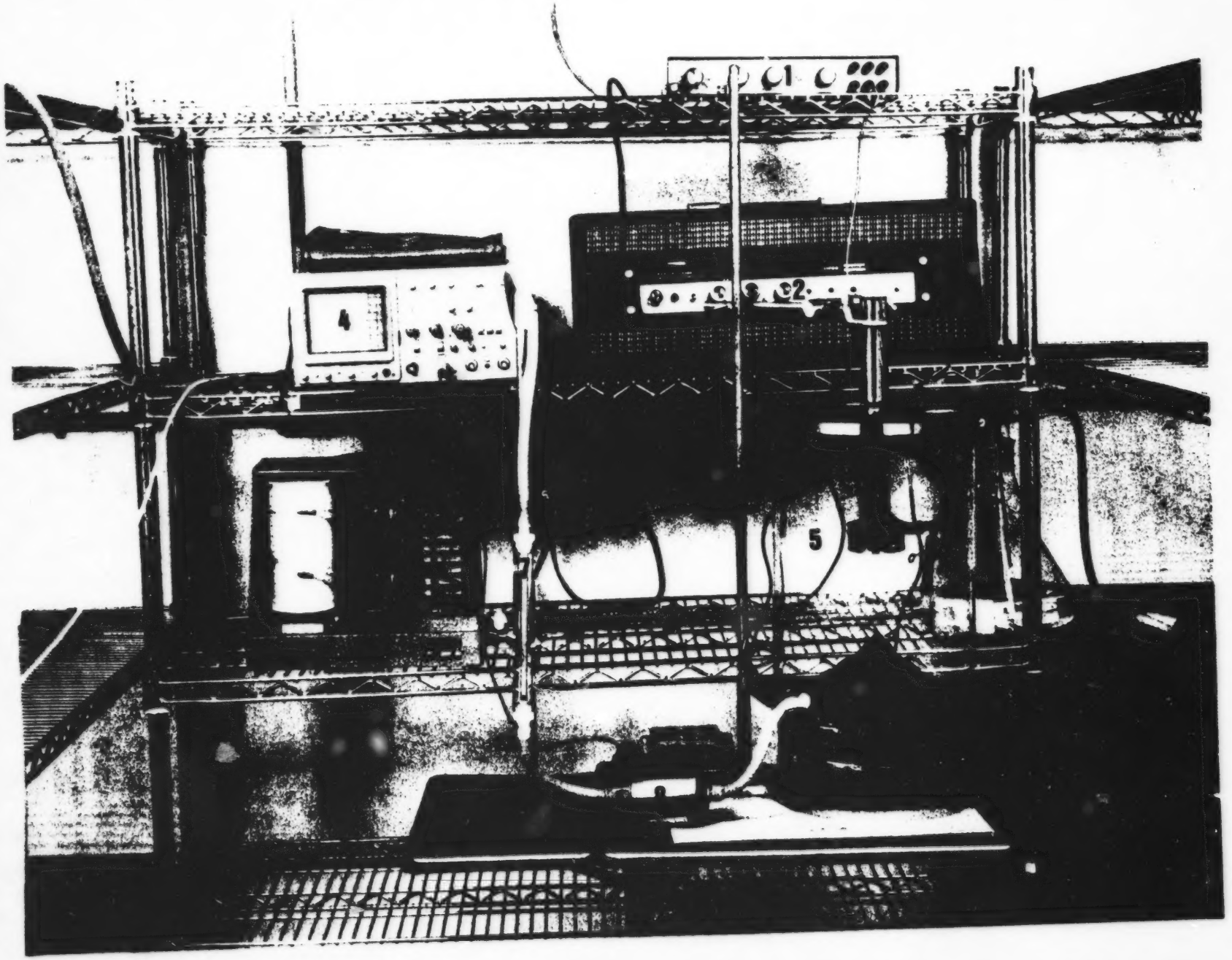

4
5

Figure 2 Paecilomyces varioti $y = 1.75X - 1275$, $r = 0.8779$

Colony forming units of Paecilomyces varioti obtained by the RCS sampler versus colony forming units added.

PAECILOMYCES VARIOTI

COLONY FORMING UNITS

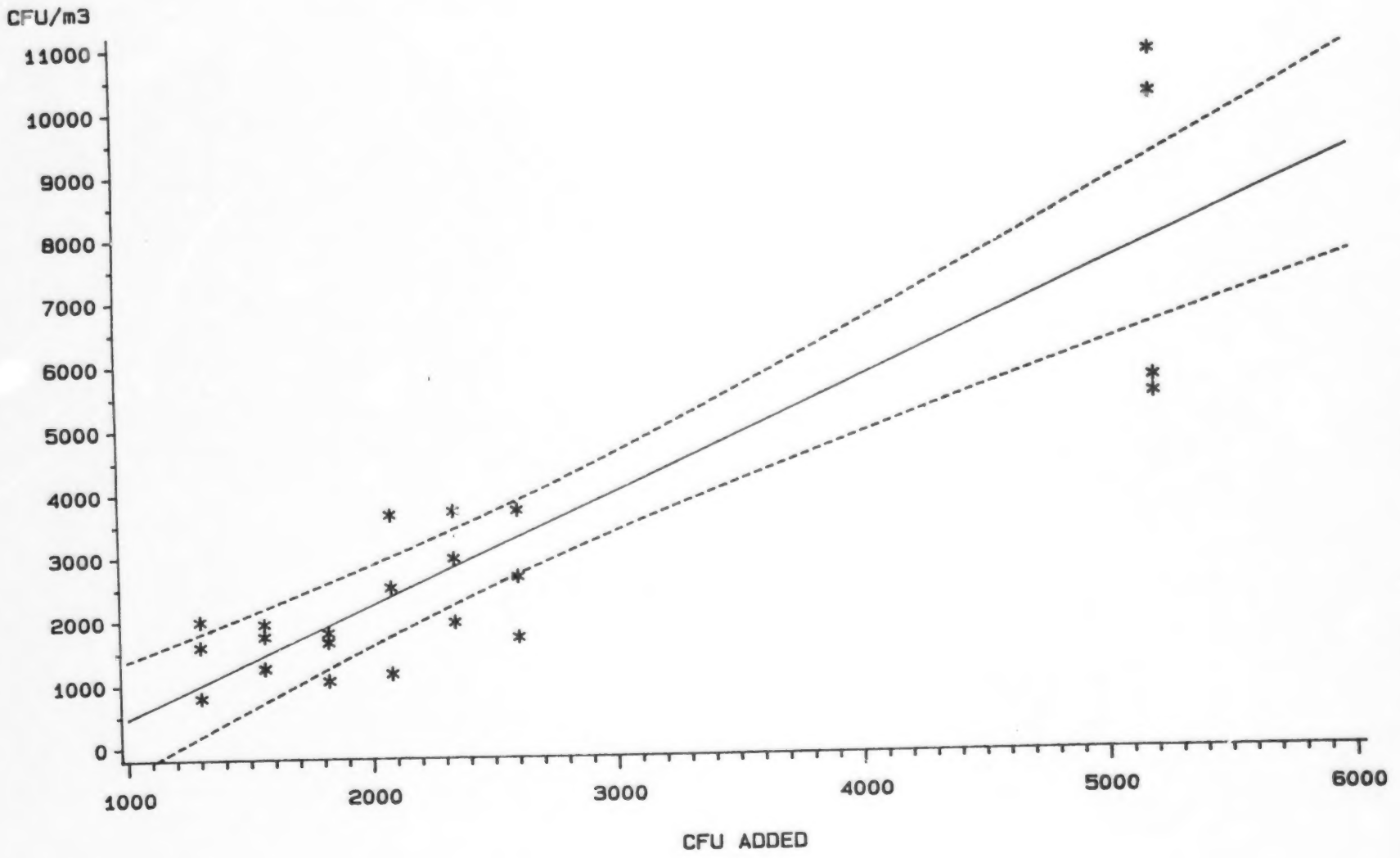


Figure 3 Penicillium viridicatum $y = 1.26X - 38.40$, $r = 0.9039$

Colony forming units of Penicillium viridicatum obtained by the RCS sampler versus colony forming units added.

PENICILLIUM VIRIDICATUM

COLONY FORMING UNITS

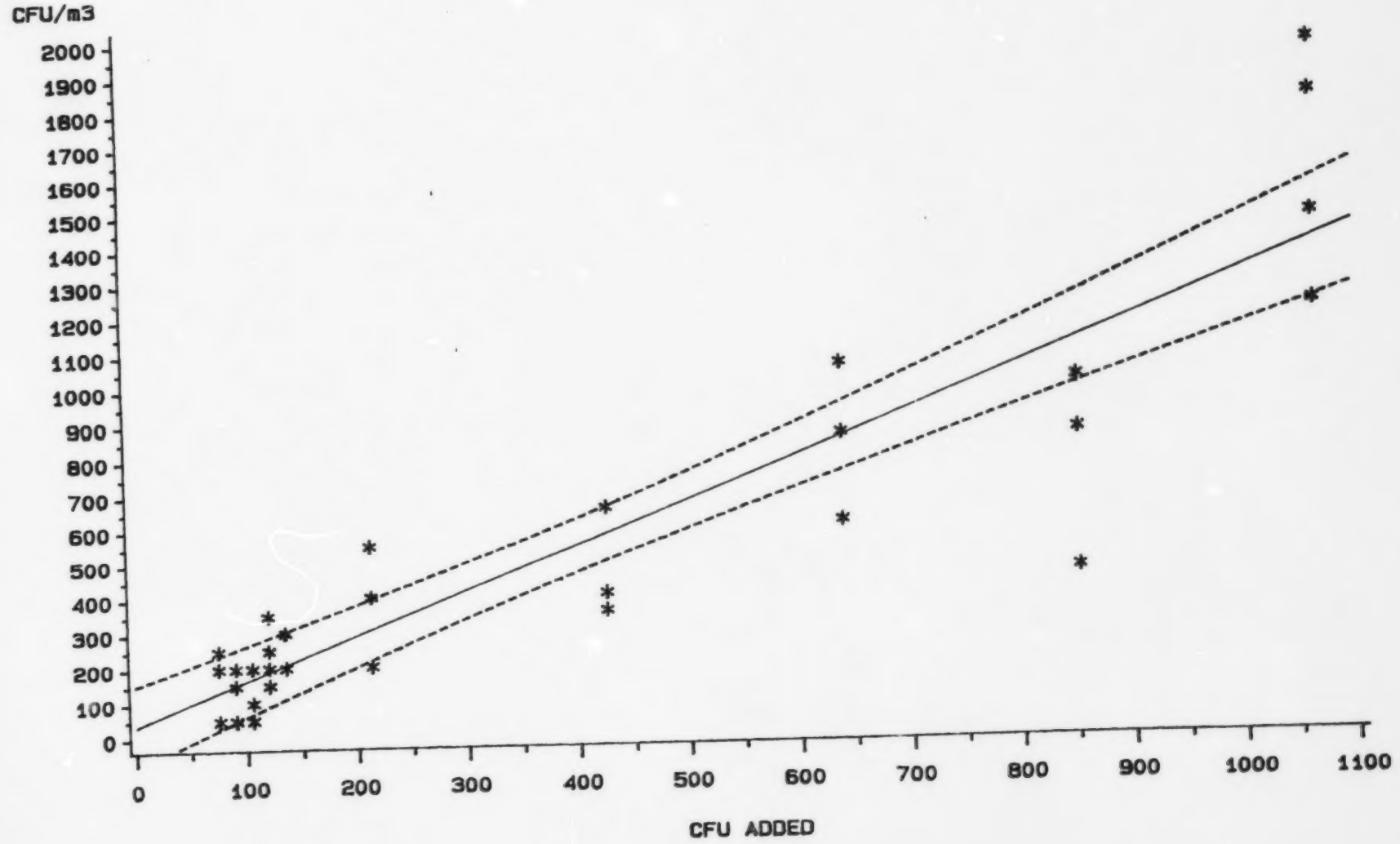


Figure 4 Alternaria alternata $y = 2.98X + 135.95$, $r = 0.9829$

Colony forming units of Alternaria alternata obtained by the RCS sampler versus colony forming units added.

ALTERNARIA ALTERNATA

COLONY FORMING UNITS

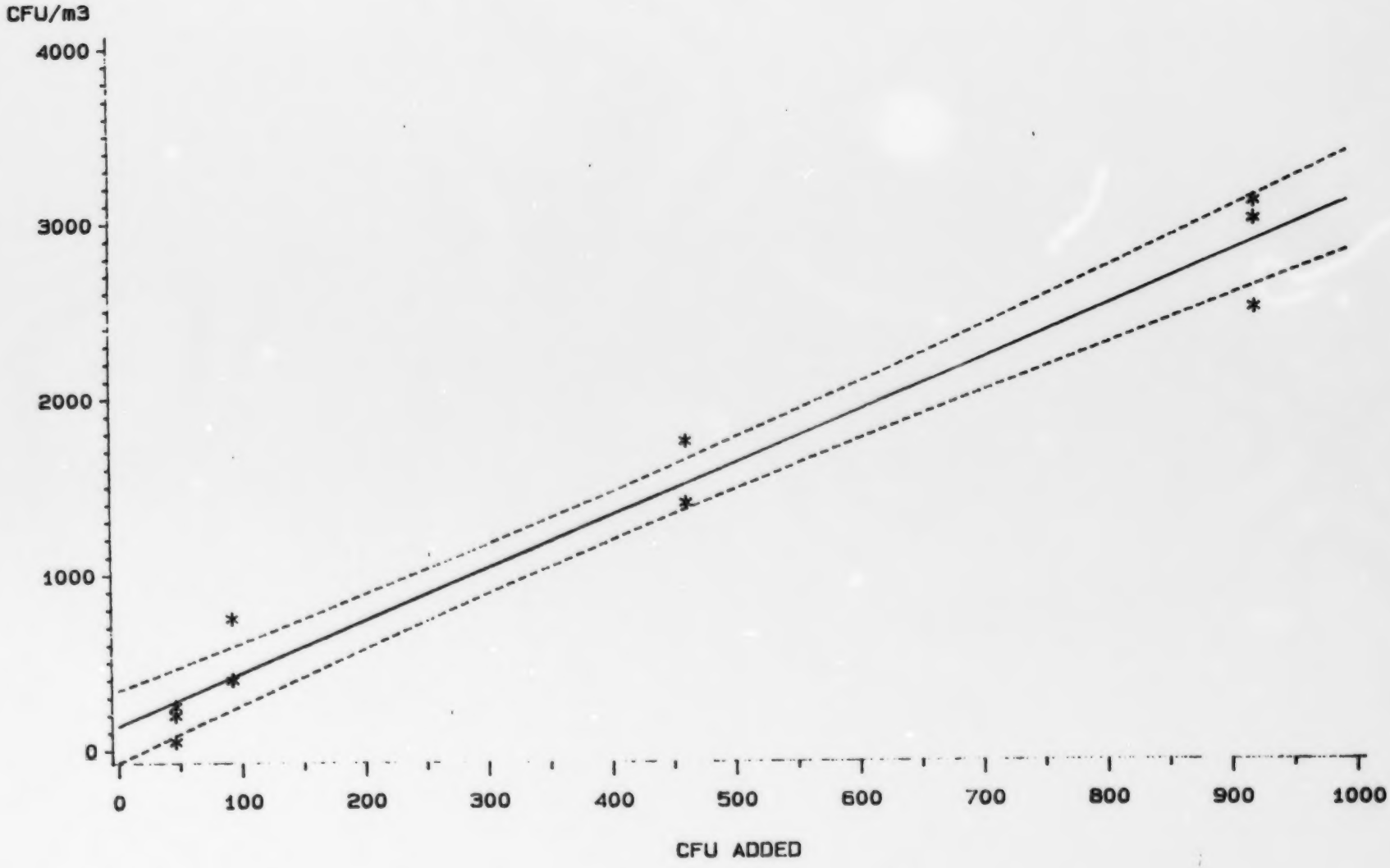


Figure 5 Cladosporium cladosporioides $y = 3.83X + 91.73$, $r = 0.9032$

Colony forming units of C. cladosporioides obtained by the RCS sampler versus colony forming units added.

CLADOSPORIUM CLADOSPORIOIDES

COLONY FORMING UNITS

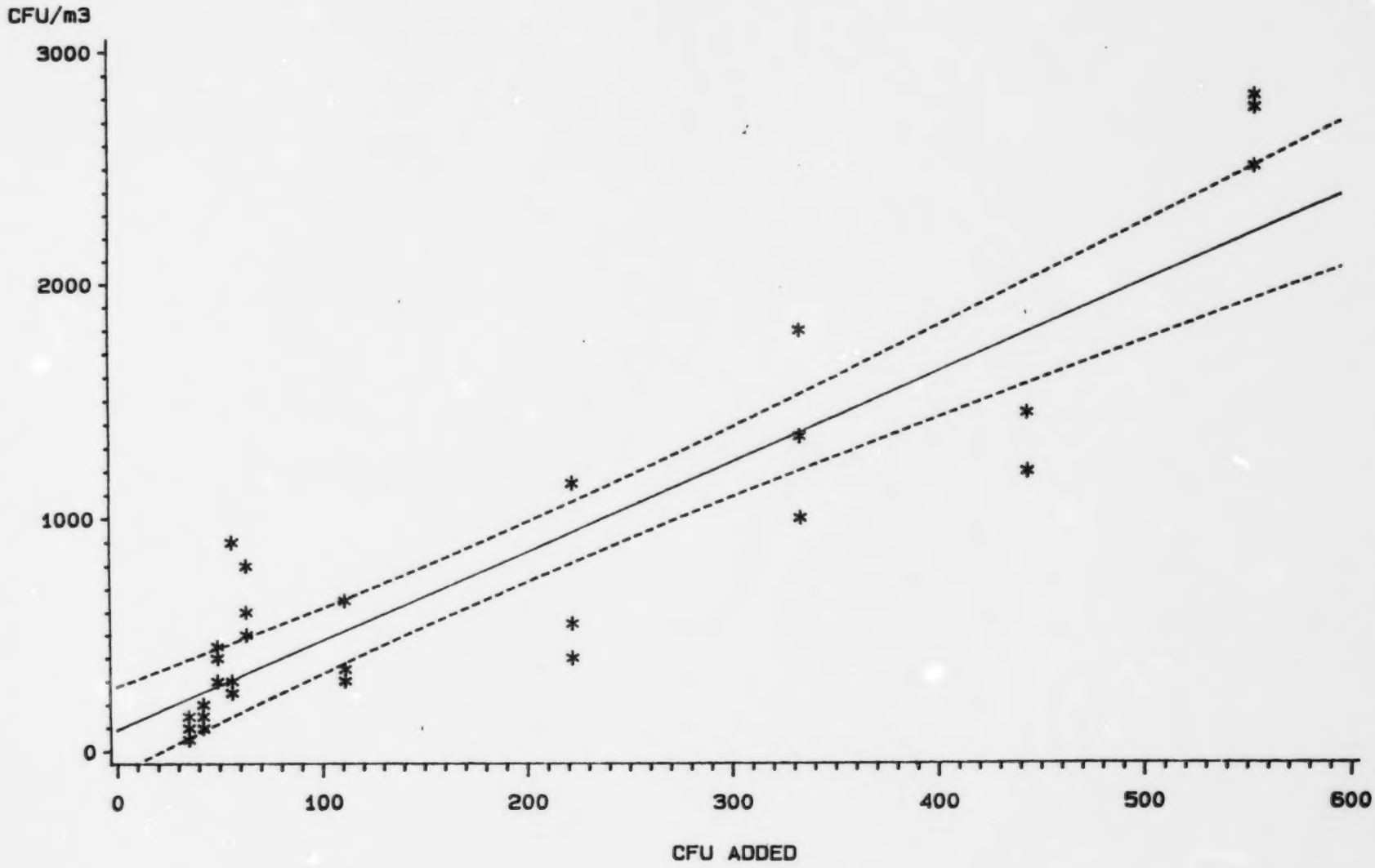
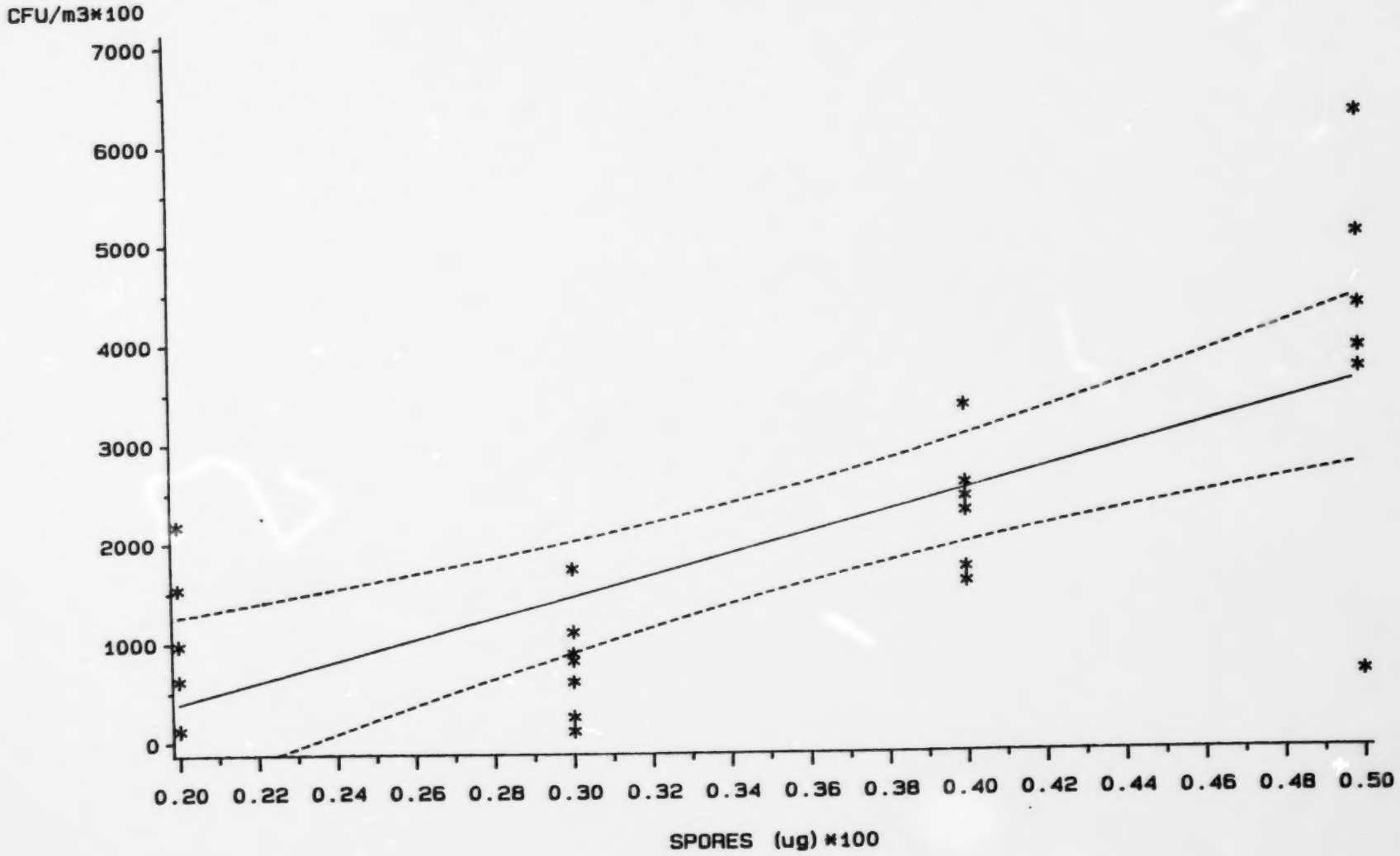


Figure 6 Cladosporium cladosporioides $y = 10602X - 1730$
$r = 0.7122$

Colony forming units of C. cladosporioides obtained by the Royco Particle counter versus the weight of spores used in ug.

CLADOSPORIUM CLADOSPORIOIDES

COLONY FORMING UNITS WITH THE ROYCO PARTICLE COUNTER



Acknowledgements:

We would like to express our appreciation of the contribution of Dr. J. D. Miller of the Plant Research Centre, Agriculture Canada for his guidance throughout the course of this work.

Literature:

1. Fradkin, A., Sampling of Microbiological Contaminants in Indoor Air. American Society for Testing and Materials. Philadelphia, 1987. pp. 66-77.

2. Delmore, R.P., W.N. Thompson. A comparison of air sampler efficiencies. Medical Device and Diagnostic, Feb. 1981, pp. 45-53.

3. Burge, H.O., Personal communication to A. Ballon, Public Works Canada, 1987.

4. A.P. Verhoeff, J.H. Von Wijoren, P. Attwood, J.S.M. Boleij, B. Brunekreel, E.S. Von Keenen and R. Samson. 1988. Enumeration and identification of airbourne mould propaguls in houses: a field comparison of selected techniques. In: Indoor and ambient air quality. Edited by R. Perry and P.W.W. Kirk. Selper Ltd. London. pp. 453-460.

5. Placencia, J.T. Peeler, J.S. Oxborrow and J.W. Danialson. 1982. Comparison of bacterial recovery by Reuter Centrifugal Air sample and slit-to-agar sampler. Appl. Environ. Microbiol. 44: 512-513.

6. Clark, S., V. Lach and O.M. Lidwell. 1981. The performance of the Biotest RCS centrifugal air sampler. J. Hosp. Infect. 2: 181-186.

7. Macher, J.M., and M.W. First. 1983. Reuter centrifugal air sampler: measurement of effective airflow rate and collection efficiency. Appl. Environ. Microbiol. 45: 1960-1962.

8. Fraser, D.G. V.A. Robinson, K. Jayaraman, K.C. Weber and D.S. Delong, C. Glance. Improved operating parameters for the Pitt-3 aerosol generator during resuspension of respirable cotton dust. Proceedings of the Tenth Cotton Dust Research Conference. Beltwide Cotton Research Conferences. Las Vegas, Nevada, January

8-9, 1986 pp. 122, 125.
9. Sorenson, W.G., D.G. Frazer, B.B. Jarvis, J. Simpson and V.A. Robinson. 1987. Trichothecene mycotoxins in aerosolized conidia of Stachybotrys Atra. Applied and Environmental Microbiology. pp. 1370-1375.